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Dr. Lori White
NTP Office of Liaison, Policy and Review
NIEHS, P.O. Box 12233, MD K2–03
Research Triangle Park, NC 27709

9 June, 2011

Dear Dr. White:

After reviewing the BG1Luc ER TA Agonist and Antagonist Protocols, the scientists at CertiChem, Inc. believe this cell line has excellent utility. We fully support and appreciate the tremendous effort put forth by the ICCVAM/NICEATM team led by Dr. Stokes and Dr. Casey.

While this is a strong assay, we have recommendations that should allow the BG1Luc ER TA assay to be more versatile. We propose changing various aspects of the protocol to allow efficient implementation by a liquid handling system. While these changes should not be significant alterations to the protocol, they could greatly increase throughput. Specifically, some liquid handling systems used to automate (roboticize) assays are incapable of vertical serial dilutions in the Y-axis, so a plate design that can accommodates serial dilutions in the X-axis is preferred. Additionally, the option to use ICI 182,780, instead of Raloxifene HCl in some assays would offer further improvement. Requested changes for both the agonist/antagonist protocols are in four general areas:

1) Allow flexible plate template design and orientation
2) Allow increased number of replicates of weak positive (Methoxychlor/Tamoxifen) & negative controls
3) Allow increased number of concentrations for strong positive controls (estradiol/raloxifene): recommend to use 8 concentrations in Range-finder experiments and 12 concentrations in comprehensive experiments
4) - Allow serial dilution for Range-finder and Comprehensive experiments to be performed in 2% dimethylsulfoxide (DMSO) in estrogen-free medium (EFM) (for example, allow serial dilutions in 2% DMSO EFM to be overlaid on 100uL cell seeding volume to achieve final 200uL volume and 1% DMSO)

These recommendations are given more specifically in the tables below.

Table entries refer to version 25 January 2011 DRAFT BG1Luc ER TA Performance Standards: Appendix B (Agonist Protocol) by line numbers

Document Section	Line Number	Current Protocol	Roboticized Protocol	Recommended Modification
6.1	144-147	7-point serial dilution for Range Finder	8-point RF and serial dilutions that run horizontally, rather than vertically on the 96-well plate	Allow the use of a bigger range of testing concentration to help identify the right concentrations for strong ER agonists
8.2	248-257	Estrogen-Free DMEM Medium	Estrogen-free RPMI medium	Allow the use of any phenol red-free medium, especially since cells are maintained already in RPMI medium
8.2	248-257	4.5% charcoal/dextran treated FBS	Combination of charcoal/dextran treated FBS and calf serum	Allow the replacement or supplementation of medium with varying percentage of FBS and/or calf serum to decrease the background
9.2	489-501	200,000 cells/mL for seeding 40,000 cells per well in 200 uL	Seed 40,000 cells/well in 100 uL	Allow 100 uL seeding volume so that treatments may be overlaid without firstly removing the seeding medium
10.1	510-525	Solubility testing in 4 mL conical tubes	Solubility testing in various sized microcentrifuge tubes and glass vials	Allow the use of any appropriately sized microcentrifuge or glass vessel to be used for solubility testing
10.2	535-645	Preparations of controls and test substances and serial dilutions (for Range Finder and Comprehensive assays) are performed in glass tubes and with very specific volumes.	Serial dilutions are performed in 96-well Deep Well Plates that are amenable to roboticized procedure.	Allow serial dilutions in any type of vessel and with smaller or larger volumes, so long as DMSO is used and specified dilution is achieved.
11.4	741-746	Injecting luminometer is used	Luciferase assay reagent is dispensed by liquid handling system and not by luminometer injectors	Allow dispensing of luciferase assay reagent by liquid handling system to speed up the plate measurement.
12	912-917	7-point serial dilution for Range Finder	8-point RF and serial dilutions that run horizontally, rather than vertically on the 96-well plate	Allow the use of a bigger range of testing concentration to help identify the right concentrations for strong ER agonists

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Table entries refer to version 25 January 2011 DRAFT BG1Luc ER TA Performance Standards: Appendix C (Antagonist Protocol) by line numbers

Document Section	Line Number	Current Protocol	Roboticized Protocol	Recommended Modification
5.2	96-107	Fixed 17beta-estradiol (E2) testing concentration	E2 concentration is based on historical comprehensive testing for E2 and approximates E2 EC80.	Allow modification of E2 concentration, as-needed, since cellular responses my drift over time and it is necessary to achieve an appropriate level of E2 activity.
5.2	96-107	Flavone is used (as is tamoxifen)	Use dibenzathrancene (DBA) -	Allow the use of tamoxifen as already allowed in changes made to the BG1 assay
6.1	143-147	7-point serial dilution for Range Finder	8-point RF and serial dilutions that run horizontally, - rather than vertically on the 96-well plate -	Allow the use of a bigger range of testing concentration to help identify the right concentrations for strong ER antagonists
8.2	244-254	Estrogen-Free DMEM Medium	Estrogen-free RPMI medium -	Allow the use of RPMI phenol red-free medium, especially since cells are maintained already in RPMI medium
8.2	244-254	4.5% charcoal/dextran treated FBS	Combination of charcoal/dextran treated FBS and calf serum	Allow the replacement or supplementation of medium with varying percentage of FBS and/or calf serum to decrease the background luciferase activity
9.2	472-479	200,000 cells/mL for seeding 40,000 cells per well in 200 uL	Seed 40,000 cells/well in 100 uL	Allow 100 uL seeding volume so that treatments may be overlaid without firstly removing the seeding medium
10.1	492-505	Solubility testing in 4 mL conical tubes	Solubility testing in various sized microcentrifuge tubes and glass vials	Allow the use of any appropriately sized microcentrifuge or glass vessel to be used for solubility testing
11.0 to 12.4	508-636	Preparations of controls and test substances and serial dilutions (for Range Finder and Comprehensive assays) are performed in glass tubes and with very specific volumes.	Serial dilutions are performed in 96-well Deep Well Plates that are amenable to roboticized procedure.	Allow serial dilutions in 96-well Deep Well Plates and with smaller or larger volumes, so long as DMSO is used and specified dilution is achieved.
14		7-point serial dilution for Range Finder	8-point RF and serial dilutions that run horizontally, rather than vertically on the 96-well plate	Allow the use of a bigger range of testing concentration to help identify the right concentrations for strong ER antagonists

Again, we appreciate all the work that the ICCVAM/NICEATM team is doing and we are excited about the - opportunity to provide our input in this review process. Please do not hesitate to ask for any clarification or - assistance. -

Sincerely, -

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